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A new species of sinistral flatfish of the genus *Chascanopsetta* (Teleostei: Bothidae) from off Papua New Guinea, western Pacific Ocean

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Abstract

Left-eyed flounders of the genus *Chascanopsetta* Alcock 1894 (Bothidae) occur in the Indian, Pacific, and Atlantic oceans at depths ranging from 120 to 1500 meters. They possess some unique features in bothid fishes including a strongly compressed and elongated body and a tremendously large mouth. Currently, nine species of *Chascanopsetta* are recognized, and three of them (*C. micrognatha* Amaoka & Yamamoto 1984, *C. lugubris* Alcock 1894 and *C. prognatha* Norman 1939) are distributed in the West Pacific. We collected 25 specimens of *Chascanopsetta* during 11 biodiversity expeditions carried out mainly in the West Pacific. Among them, eight specimens taken off Papua New Guinea present morphological features that differ from those of the three nominal species known in the West Pacific. In this study, we examined these eight specimens of unknown affinity and compared their morphology to that of specimens of other congeneric species. Results of these comparisons showed that these specimens represent an undescribed species of *Chascanopsetta*, named herein, *C. novaeguineae* **sp. nov.**. The new species resembles *C. elski* Foroshchuk 1991, which is known only from the Saya de Malha Bank in the western Indian Ocean, in having a high number of gill rakers (> 13). However, the combination of the following characters further distinguishes *C. novaeguineae* **sp. nov.** from *C. elski*: longer jaws, narrower interorbital width, and number of pseudobranches (21–25 vs. 26–27). The DNA sequences from the mitochondrial cytochrome oxidase subunit I (*COI*) gene from *C. novaeguineae* **sp. nov**. and other species were obtained and compared to confirm its taxonomic status and to infer its tentative phylogenetic position within the *Chascanopsetta*.

Key words: Pleuronectiformes, Bothidae, *Chascanopsetta*, new species, Papua New Guinea

Introduction

The sinistral flatfish genus *Chascanopsetta* Alcock 1894 (Pleuronectiformes, Bothidae) occurs on the deep-sea bottom at depths ranging from 120 to 1500 meters in the Atlantic, Indian, and Pacific Oceans (Bruun 1937; Amaoka & Parin 1990; Foroshchuk 1991). Species of *Chascanopsetta* possess some adaptive features unique in bothid fishes as a result of their particular life style in deep-water habitats. The genus *Chascanopsetta* is easily distinguished from other bothid genera by the following characteristics: an enormously large mouth, lower-jaw tips projecting beyond the upper-jaw tips, lateral lines developed on both sides of the body, and a greatly compressed body (Amaoka & Yamamoto 1984).

Nine species of the genus *Chascanopsetta* have been recognized as valid species including: *C. lugubris* Alcock 1894, *C. crumenalis* (Gilbert & Cramer 1897), *C. prorigera* Gilbert 1905, *C. danae* Bruun 1937, *C. prognatha* Norman 1939, *C. micrognatha* Amaoka & Yamamoto 1984, *C. megagnatha* Amaoka & Parin 1990, *C. elski* Foroshchuk 1991, and *C. kenyaensis* Hensley & Smale 1997 (Amaoka & Yamamoto 1984; Amaoka & Parin 1990; Foroshchuk 1991; Hensley & Smale 1997). Among them, *C. micrognatha*, *C. lugubris* and *C. prognatha* occur in the western Pacific region (Amaoka & Yamamoto 1984; Amaoka & Parin 1990; Hensley & Amaoka 2001; Munroe 2003).

Amaoka & Yamamoto (1984) first reviewed and provided an identification key of species of this genus in a paper describing their new species, *C. micrognatha*. The taxonomy of species of this genus was subsequently

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revised by the following studies: Foroshchuk (1991) with the description of *C. elski* based on the specimens collected from the Saya de Malha Bank in the western Indian Ocean; and Hensley & Smale (1997) with the description of *C. kenyaensis* based on five specimens collected from the coasts of Kenya and southern Somalia. According to previous studies (Amaoka & Yamamoto 1984; Foroshchuk 1991; Hensley & Smale 1997; Munroe 2003), species of the genus *Chascanopsetta* can be separated into two species groups based on the number of gill rakers on the lower limb of the first gill arch. Members in the first species group (*C. crumenalis*, *C. danae*, *C. lugubris*, *C. megagnatha*, and *C. prognatha*) have less than six gill rakers, while members in the second species group (*C. elski*, *C. kenyaensis*, *C. microgna*tha, and *C. prorigera*) have 8–19 gill rakers.

During 11 biodiversity expeditions carried out between 2007 and 2017 mainly in the West Pacific under the *Tropical Deep-Sea Benthos* (*TDSB*) program and the cooperative project between Taiwan and France entitled "Taiwan-France marine diversity exploration and evolution of deep-sea fauna (TFDeepEvo), we collected eight undescribed specimens of *Chascanopsetta* in deep waters off Papua New Guinea. All of the undetermined specimens belong to the second species group, i.e., those species with more than 13 gill rakers on the lower limb of the first gill arch. After a detailed examination and comparison with morphologically similar congeners (i.e., *C. elski*), we concluded that these unknown specimens represent an undescribed species of the genus *Chascanopsetta*. The purpose of the present work is to formally describe this new species.

Materials and methods

A total of 25 specimens belonging to two of the nine currently recognized species and a previously unrecognized species of *Chascanopsetta* were collected during the following biodiversity expeditions: AURORA 2007, SALOMONBOA 3, BIOPAPUA 2010, MADEEP, HanHai 2014, DongSha 2014, KAVIENG 2014, ZhongSha 2015, KARUBENTHOS 2015, KANACONO, and KANADEEP. The specimens were collected from deep waters off Guadeloupe Island, New Caledonia, Papua New Guinea, Philippines, Solomon Islands, the South China Sea, and from off Taiwan (Fig. 1). A small piece of muscle tissue was taken from fresh specimens and preserved in 95% ethanol to obtain their mitochondrial cytochrome oxidase subunit I (*COI*) gene sequences in order to further discriminate and characterize the genetic identity of the new species. Collected specimens were photographed before fixation in 10% formaldehyde and then transferred into 70% ethanol for long-term preservation. Methods for counting meristic features and for taking morphometric measurements followed those outlined in Amaoka & Yamamoto (1984) and Foroshchuk (1991). The abbreviation and definition of morphometric measurements in this study were defined as follows: standard length (SL), length measured from tip of mandible to the end of hypural plate; head length (HL), length measured from tip of mandible to the most posterior extension of upper operculum lobe; body depth (BD), vertical distance measured at the deepest part of body from dorsal margin to ventral margin; snout length (SNL), distance measured from tip of snout to anterior rim of lower eye; upper jaw length on ocular side (UJOL), shortest distance measured from bony tip of premaxilla to the posterior tip of maxilla on ocular side; upper jaw length on blind side (UJBL), shortest distance measured from bony tip of premaxilla to the posterior tip of maxilla on blind side; mandible length on ocular side (MOL), shortest distance measured from bony tip of mandible to the end point of lower jaw on ocular side; mandible length on blind side (MBL), shortest distance measured from bony tip of mandible to the end point of lower jaw on ocular side; upper eye diameter (UED), greatest diameter of the upper eye measured; lower eye diameter (LED), greatest diameter of the lower eye measured; Interorbital width (IOW), shortest distance measured between bony edges of eyes; pectoral-fin length on ocular side (PEOL), longest length of pectoral fin measured on ocular side; pectoral-fin length on blind side (PEBL), longest length of pectoral fin measured on blind side; pelvic-fin length on ocular side (PLOL), longest length of pelvic fin measured on ocular side; pelvic-fin length on blind side (PLBL), longest length of pelvic fin measured on blind side; lateral-line curve length on ocular side (LCL), length measured from the starting point of lateral-line curve to its end; lateral-line curve depth on ocular side (LCD), distance measured at the deepest part of lateral-line curve; and caudal peduncle depth (CPD), vertical distance measured at the narrowest part of caudal peduncle. In addition, all adult specimens representing the undetermined species were radiographed in order to observe dorsal-fin and anal-fin pterygiophores and vertebrae. Maturity and sex were determined by macroscopic examination. Mature females were confirmed by observing an extent of posterior elongation of ovaries for the presence of apparent developed oocytes in the ovaries under light transmitted through the body. As there is no

obvious difference in testes size between mature and immature males in *Chascanopsetta* species, the maturity can only be determined in female specimens by our examination.

Total genomic DNA was extracted from 26 individuals (including one outgroup, *Taeniopsetta ocellata*) and 13 *COI* reference sequences were retrieved from GenBank (including two additional bothid outgroups, *Trichopsetta ventralis* and *Monolene sessilicauda*) (Table 1). The DNA extraction was performed using the LabTurbo–Automated DNA/RNA Extraction System and LabTurbo DNA Mini Kit LGD480–220 (Taigen Bioscience Corporation, Taipei, Taiwan) following manufacturer protocols.

COI gene sequences were amplified using universal primers (Ward *et al.* 2005) and specific primers for flatfishes: Flatfish-COIF 5'-TCR ACC AAY CAC AAA GAC ATY GGC AC3-' and Flatfish-COIR 5'-TAY ACY TCT GGG TGR CCA AAG AAT CA3-'. A polymerase chain reaction (PCR) was carried out using a thermal cycler (Applied Biosystems) in 25 µL reaction volumes contained 12.9 µL sterile distilled water, 5.0 µL of 5x Go Tag Flexi buffer reaction buffer, 5.0 µL of MgCl (25 mM), 2 µL of Deoxynucleotide Triphosphate (dNTPs), 0.5 µL of each primer (10 uM), 0.1 µL of Taq polymerase (Genomics), and 2 µL of template DNA. The thermal cycle profile was set as follows: denaturation at 94˚C for 1 minute, followed by 35 cycles of denaturing at 94˚C for 30 seconds, annealing at 51˚C for 30 seconds and extension at 72˚C for 40 seconds, with a final extension at 72˚C for 3 min. Gel electrophoresis was performed to visualize the PCR products using 1% agarose gel (1x TBE buffer) under ultraviolet light. Purified PCR products were applied to the Center of Biotechnology, National Taiwan University for sequencing with the Sanger method.

Sequence chromatograms were edited using CodonCode Aligner v.7.1.2 (CodonCode Corporation, Dedham, MA, USA). DNA sequences (length range from 524 to 651 bp) were manually aligned using the sequence alignment editor Se-Al v2.0a11 (Rambaut 1996). Aligned *COI* sequences were then analyzed in a maximum likelihood (ML) framework partitioned by codon position. The general time reversible (GTR) nucleotide substitution model with gamma-distributed rate variation (G) was applied to each partition and analysis conducted with the Randomized Axelerated Maximum Likelihood method in RAxML v. 8.0.4 (Stamatakis 2014). Nodal support was assessed with 1000 bootstrap replicates (Felsenstein 1985). Genetic differences between species were estimated by pairwise genetic distances among the examined samples. The genetic distance was set to a Kimura two-parameter (K2P) corrected distance and calculated using MEGA v7.0 (Kumar *et al.* 2016).

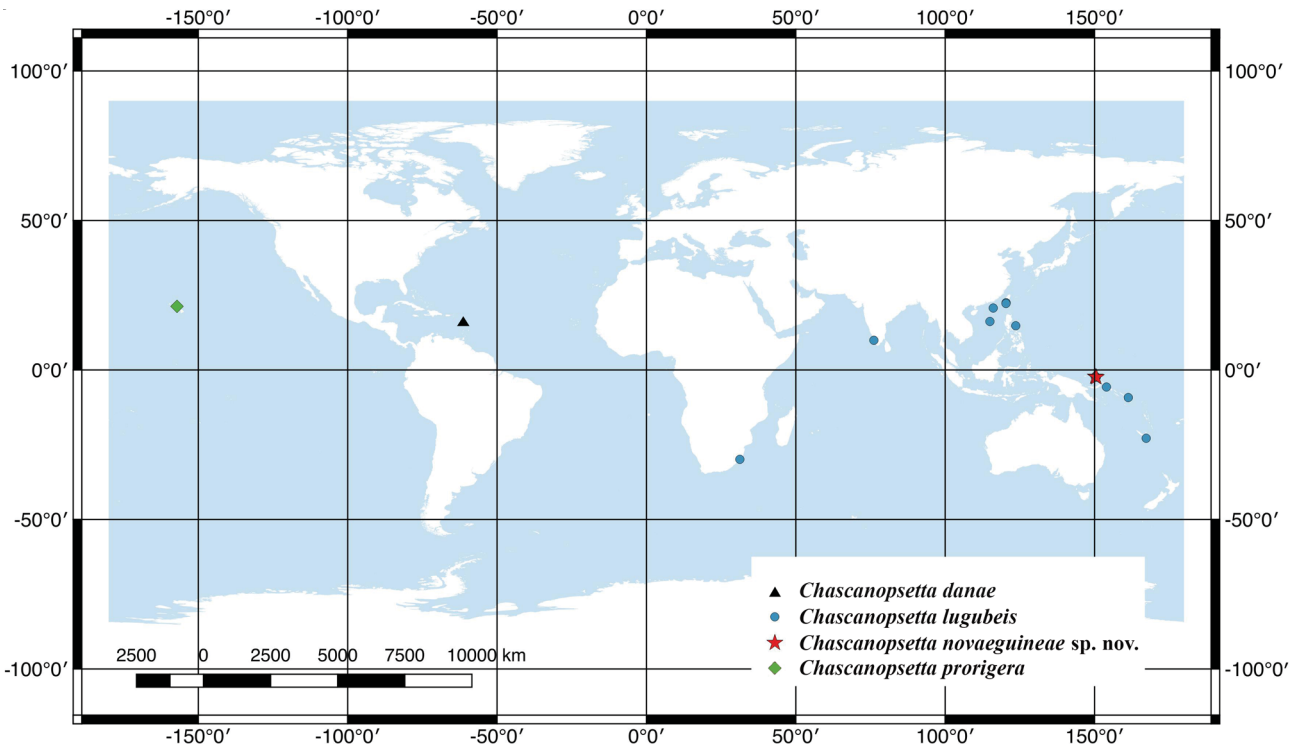

FIGURE 1. Sample localities of four species of the genus *Chascanopsetta* examined in this study: *C. danae* (triangle), *C. lugubris* (circle), *C. novaeguineae* **sp. nov.** (star), and *C. prorigera* (rhombus).

TABLE 1. Taxa, collection information, and GenBank accession numbers for the *COI* gene sequences of the *Chascanopsetta* used in this study. Number sign (#), outgroup; asterisk (*), erroneous information on species name or sample locality; NA, not available; accession numbers in bold letters, sequences obtained in this study.

Taxon/Sample no.					GenBank accession no.
	Voucher specimens	Tissue voucher	Locality	Depth (m)	COI
Monolene sessilicada#	06-412	NA	Canada	NA	KC015695
Trichopcetta ventralis#	DPND 1903	NA	USA, Gulf of Mexico	NA	MF041352
Taniopsetta ocellata#	NTUM 14120	WJC 6146	South China Sea	190–221	**MG865864**
Chascanopsetta danae	NA	WJC 6959	Caribbean Sea	251–416	**MG865865**
Chascanopsetta lugubris	NA	NA	Japan	NA	AP017455
Chascanopsetta lugubris	Smith 259.6 #2_05	NA	South Africa	NA	JF493114
Chascanopsetta lugubris	Smith 259.6 #5_05	NA	South Africa	NA	JF493115
Chascanopsetta lugubris	Smith 259.6 #3_05	NA	South Africa	NA	JF493116
Chascanopsetta lugubris	NA	NA	China	NA	KJ433561
Chascanopsetta lugubris	NBFGR:CHN:17	NA	India	NA	KP244514
Chascanopsetta lugubris	NBFGR:CHN:AK110	NA	India	NA	KP244515
Chascanopsetta lugubris	NBFGR:CHN:AK111	NA	India	NA	KP244516
Chascanopsetta lugubris	ASIZP 64870	ASIZP 800419	Taiwan	NA	KU892989
Chascanopsetta lugubris	NA	NA	China	NA	NC033392
Chascanopsetta lugubris	ASIZP 68026	ASIZP 913787	Philippines	367–357	**MG865873**
Chascanopsetta lugubris	ASIZP 68036	ASIZP 913797	Philippines	500–524	**MG865874**
Chascanopsetta lugubris	ASIZP 73792	ASIZP 916293	Papua New Guinea	402–440	**MG865875**
Chascanopsetta lugubris	NTUM 14114	NC 223	New Caledonia	535–563	**MG865876**
Chascanopsetta lugubris	NTUM 14114	NC 224	New Caledonia	535–563	**MG865877**
Chascanopsetta lugubris	NTUM 13513	NC 1546	New Caledonia	550	**MG865878**
Chascanopsetta lugubris	NTUM 13514	NC 1555	New Caledonia	600	**MG865879**

...continued on the next page

TABLE 1. (continued)

Taxon/Sample no.					GenBank accession no.
	Voucher specimens	Tissue voucher	Locality	Depth (m)	COI
Chascanopsetta lugubris	NTUM 11754	PNG 2009	Papua New Guinea	398–406	**MG865880**
Chascanopsetta lugubris	NTUM 10038	PNG 2618	Papua New Guinea	470–525	**MG865881**
Chascanopsetta lugubris	NTUM 11108	PNG 2723	Papua New Guinea	685–824	**MG865882**
Chascanopsetta lugubris	NA	SB12	Solomon Islands	120–175	Unpublished from A. Dettaï (MNHN)
Chascanopsetta lugubris	NTUM 14115	WJC 2873	South China Sea	310–346	**MG865883**
Chascanopsetta lugubris	NTUM 14116	WJC 3024	South China Sea	265–300	**MG865884**
Chascanopsetta lugubris	NTUM 14117	WJC 3634	South China Sea	327–372	**MG865885**
Chascanopsetta lugubris	NTUM 14118	WJC 3874	South China Sea	420–444	**MG865886**
Chascanopsetta lugubris	NTUM 14119	WJC 6094	South China Sea	505–511	**MG865887**
Chascanopsetta lugubris	NTUM 14119	WJC 6106	South China Sea	505–511	**MG865888**
Chascanopsetta lugubris	NTUM 14121	WJC 6680	Taiwan	NA	**MG865889**
*Chascanopsetta prognatha**	FNSIC063-11	NA	South China Sea	NA	JQ681431
*Chascanopsetta lugubris**	ASIZP 73836	ASIZP 916333	Taiwan*	402–440	KU945123
Chascanopsetta novaeguineae **sp. nov.**	ASIZP 73775	ASIZP 916279	Papua New Guinea	490–505	**MG865866**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 10990	PNG 1726	Papua New Guinea	273–324	**MG865867**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 11063	PNG 2531	Papua New Guinea	335–340	**MG865868**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 14122	PNG 2561	Papua New Guinea	366	**MG865871**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 10675	PNG 2562	Papua New Guinea	366	**MG865872**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 12333	PNG 2951	Papua New Guinea	342–380	**MG865869**
Chascanopsetta novaeguineae **sp. nov.**	NTUM 12333	PNG 2952	Papua New Guinea	342–380	**MG865870**
Chascanopsetta prorigera	BPBM:FR 358	NA	Hawaii, USA	NA	DQ521029

Results

***Chascanopsetta novaeguineae* sp. nov.**
English common name: New Guinean pelican flounder
(Figures 2, 3, 4; Table 2)

Holotype. NTUM 11063 (tissue voucher: PNG 2531), mature female, 200 mm SL, sta. CP4418, 2°27'S, 150°40'E, 335–340 m, off New Hanover Island, New Ireland Province, Papua New Guinea, French beam trawl, 28 Aug. 2014, R/V *Alis*, KAVIENG 2014.

Paratypes. Five specimens, collected with the same method and from the same area as the holotype: ASIZP 73775 (tissue voucher: ASIZP 916279), mature female, 215 mm SL, sta. CP3654, 2°14'S, 150°16'E, 490–505 m, 28 August 2010, BIOPAPUA 2010; NTUM 12333 (tissue voucher: PNG 2951), sex unknown, 160 mm SL, sta. CP4445, 2°15'S, 150°17'E, 342–380 m, 1 October 2014, KAVIENG 2014; NTUM 12333 (tissue voucher: PNG 2952), unknown sex, 152 mm SL, sta. CP4445, 2°15'S, 150°17'E, 342–380 m, 1 October 2014, KAVIENG 2014; NTUM 14122 (tissue voucher: PNG 2561), mature female, 240 mm SL, sta. CP4419, 2°26'S, 150°38'E, 366 m, 28 August 2014, KAVIENG 2014; NTUM 10675 (tissue voucher: PNG 2562), sex unknown, 168 mm SL, sta. CP4419, 2°26'S, 150°38'E, 366 m, 28 August 2014, KAVIENG 2014.

Non-types. Two specimens collected with the same method and from the same area as the type specimens: ASIZP 73836 (tissue voucher: ASIZP 916333), juvenile, sex unknown, 109 mm SL, sta. CP3655, 2°15'S, 150°16'E, 402–440 m, 29 August 2010, BIOPAPUA 2010; NTUM 10990 (tissue voucher: PNG 1726), juvenile, sex unknown, 109 mm SL, sta. CP4254, 2°28'S, 150°42'E, 273–324 m, 24 April 2014, KAVIENG 2014.

Diagnosis. *Chascanopsetta novaeguineae* **sp. nov.** is characterized by the following morphological features: gill rakers on lower limb of the first gill arch 14–18; pseudobranch filaments 21–25; ratio of HL to UJOL and to UJBL 1.36–1.48, 1.39–1.51, respectively; ratio of HL to LJOL and to LJBL 1.04–1.11, 1.04–1.10, respectively; posterior flap of anterior-nostril on blind side not extending to base of posterior nostril; and small yellow blotches scattered on anterior part of ocular side of body (Fig. 3).

Description. Meristic and morphometric characters of the type series are given in Table 2. Those of the holotype are listed first, followed by the range of the paratypes placed within parentheses.

Body elongated, elliptic, and greatly compressed laterally, with ratio of SL to BD 2.93 (2.93–3.48); caudal peduncle narrow, with ratio of BD to CPD 6.14 (5.25–6.21); head relatively large, with ratio of BD to HL 0.64 (0.64–0.76). Dorsal profile of head anterior to eyes slightly concave in front of eyes, becoming convex above upper eye; snout relatively short, pointed and slightly blunt. Eyes moderately large, with ratio of HL to UED and LED 4.10 (3.22–3.71), 4.37 (3.37–4.06), respectively, and separated; lower margin of upper orbit and upper margin of lower orbit slightly elevated and ossified, no stout bony ridges in interorbital region. Nostrils on ocular side located at anterior margin of lower eye; anterior nostril tubular, with triangular flap; posterior nostril formed as a small hole; flap of anterior nostril not reaching base of posterior nostril when fully extended.

Nostrils on blind side located below origin of dorsal fin, anterior nostril tubular with a small flap posteriorly; flap of anterior nostril not reaching base of posterior nostril when fully extended; posterior nostril not tubular, forming a small hole.

Mouth oblique, extremely large. Upper jaw slightly curved; lower jaw projecting beyond upper jaw, with bony lamella along ventral margin of lower jaw (Fig. 4A). Teeth of both jaws uniserial, caniniform, depressible, becoming smaller posteriorly. Teeth on upper jaw smaller than those of lower jaw, posterior teeth of lower jaw curved inward. Gill rakers (14–18) on first gill arch on ocular side variable in size, well-developed or rudimentary without serration on ocular side; no gill rakers on upper limb (Fig. 4B).

Dorsal fin with first 3–4 rays elongate and narrowly connected by membrane only at their bases; height of succeeding rays gradually increasing in height posteriorly until about three-quarters of body length, then fin rays gradually decreasing in length to end of dorsal fin. Shape and pattern of anal-fin rays same as those of dorsal fin, except anterior rays not elongated; origin of anal fin connected to last pelvic-fin ray on ocular side with a low membrane. Base of first pelvic-fin ray on ocular side behind isthmus; base of first pelvic-fin ray of blind side located at same level of third fin ray on ocular-side pelvic fin. Pectoral fins asymmetrical, with length of blind-side pectoral fin much shorter (0.44–0.66 times in PEOL) than that on ocular side. Caudal fin rounded; all fin rays unbranched, except two uppermost and two lowermost rays of caudal fin. All fin bases scaleless except that on caudal fin.

A single lateral line presents on each side of body, slightly curved anteriorly above pectoral fin; lateral-line curve on ocular side, with ratio of HL to its length 1.78 (1.59–1.98) and to its depth 8.48 (6.67–8.48); depth of lateral-line curve much shorter, 0.21 (0.19–0.27 times in HL) than curve length. Body and head completely covered with small cycloid scales except for snout, jaws, and interorbital region.

Vent located on blind side, anterior to anal-fin origin. Genital papilla on ocular side, opposite to vent.

Coloration. Ocular side of fresh specimen (Fig. 3A): ground color of head and body yellow to reddish brown, with round black spots and small yellow blotches scattered on anterior part of body; ocular-side lateral line dark, covered with melanophores; abdominal area bluish-black due to darker peritoneal pigments obscured through abdominal wall and clearly visible externally; lips and bony lamella of jaws paler; anterior nostril yellow to light brown; pupil bluish-black, surrounded by yellow iris; dorsal half of lower eye and ventral half of upper eye with bluish tinge.

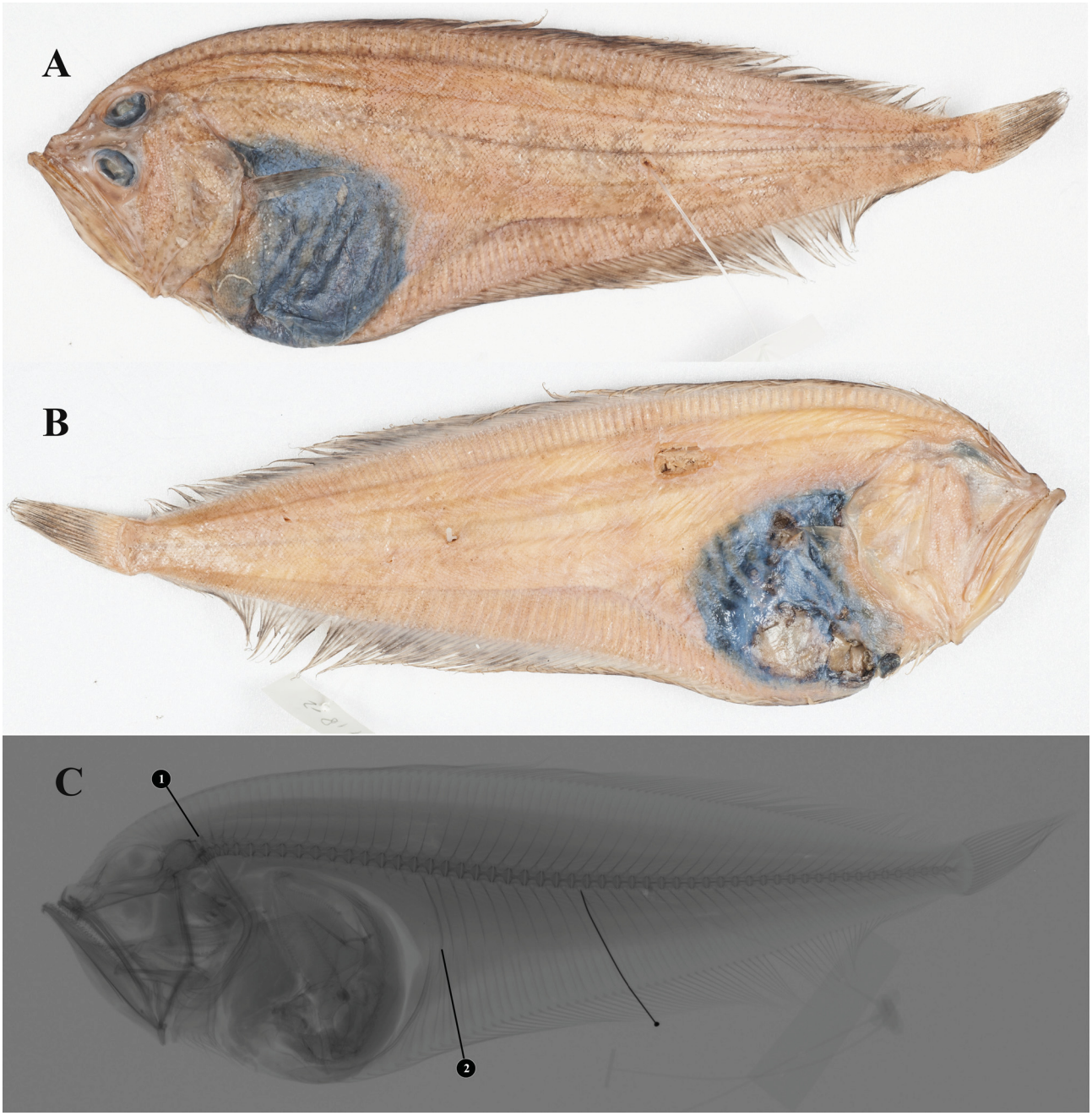

FIGURE 2. *Chascanopsetta novaeguineae* **sp. nov.**, holotype, NTUM 11063, Female, 200 mm SL, preserved specimen, from off New Hanover Island, New Ireland Province, Papua New Guinea. A. Ocular side. B. Blind side. C. Radiograph: 1, Neural spine on the second thoracic vertebra. 2, Haemal spine on the first caudal vertebra.

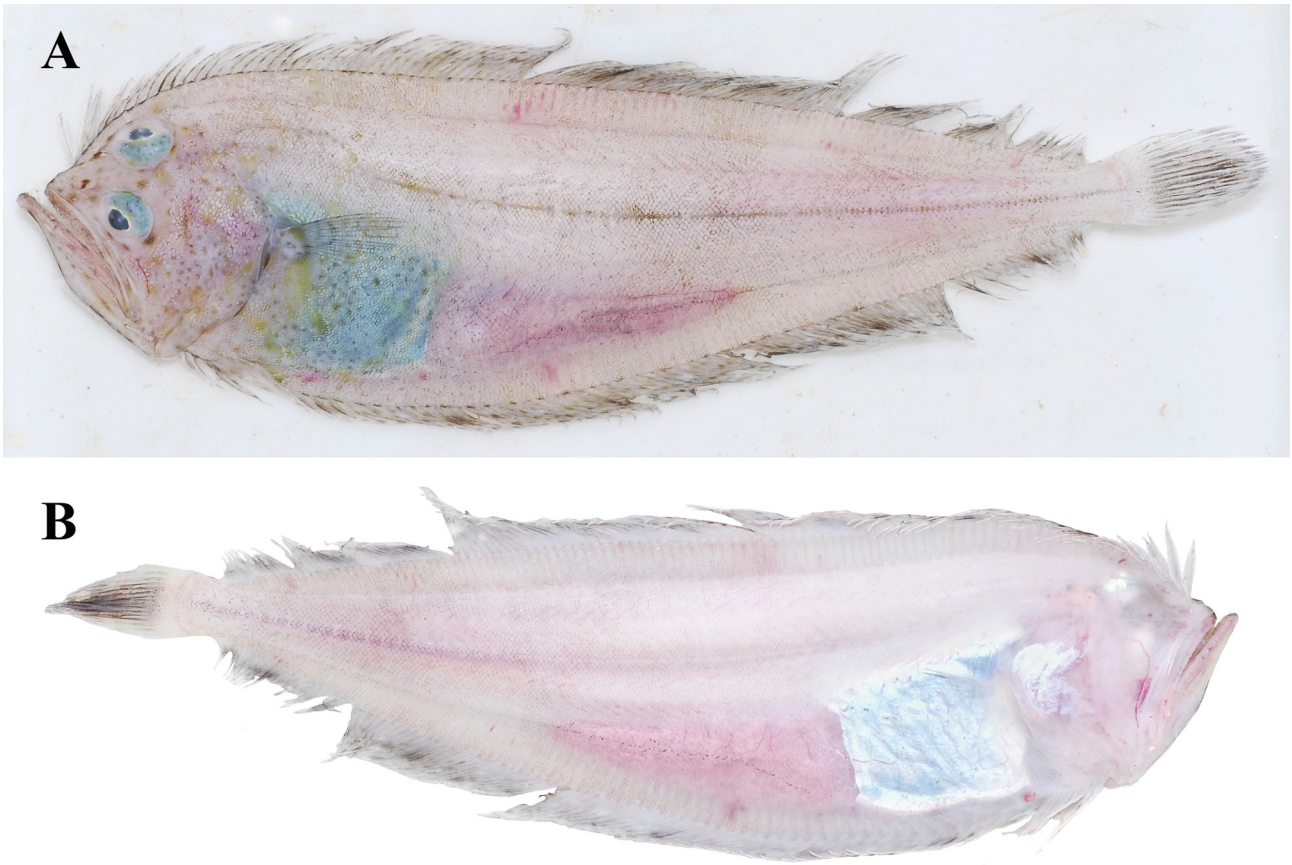

FIGURE 3. Paratype of *Chascanopsetta novaeguineae* **sp. nov.**, ASIZP 73775, (female), 215 mm SL, fresh specimen. A. Ocular side. B. Blind side.

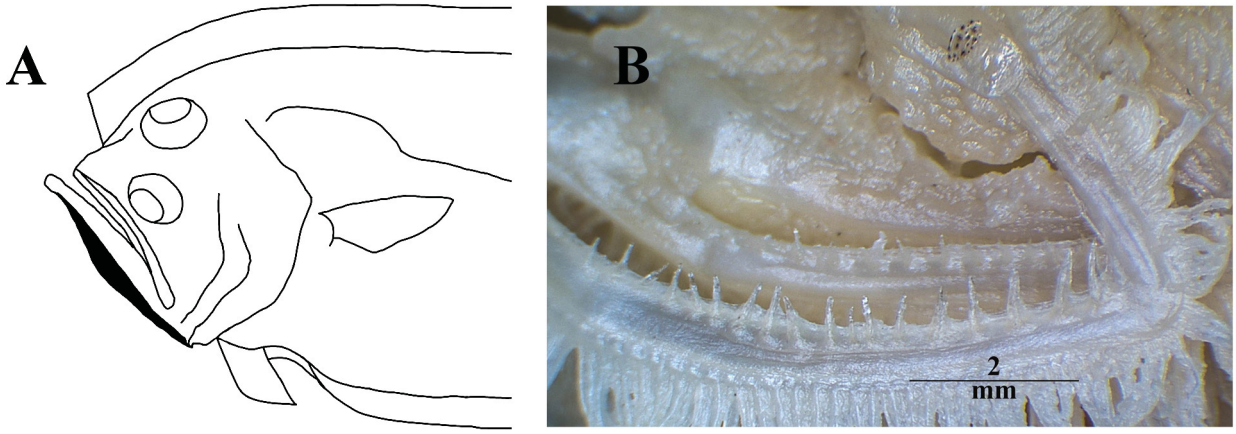

FIGURE 4. Diagnostic characters in *Chascanopsetta novaeguineae* **sp. nov.**. A. Black area showing the dilated bony lamella. B. Gill rakers on the first gill arch.

Blind side of fresh specimen (Fig. 3B): head and body uniformly white with bluish-black peritoneum observed through abdominal wall; blind-side lateral line pale and lacking pigmentation on blind side; outer and inner surfaces of opercle white to yellow, same as background coloration; dorsal, anal and pelvic fins uniformly light brown; dorsal and anal fins with small brown spots scattered throughout dorsal and anal fins; basal margins of fin rays and fin membranes on blind side white to light yellow.

Coloration of recently preserved specimen (Fig. 2A–B) is similar to that of freshly-caught fishes.

TABLE 2. Meristic features and morphometric measurements of *Chascanopsetta novaeguineae* **sp. nov**. and *C. elski*.

	C. novaeguineae **sp. nov.**		*C. elski*	
	Holotype	Paratypes (n=5)	Holotype	Paratypes(n=17)
Standard Length, SL (mm)	200.35	151.91–240.07	240	219–280
Ratio of SL to				
Head length (HL)	4.59	4.32–4.67	4.40	4.02–4.45
Body depth (BD)	2.93	2.93–3.48	3.10	2.68–3.26
Ratio of HL to				
Snout length (SNL)	4.35	4.02–5.03	3.41	3.41–4.07
Upper jaw length on ocular side (UJOL)	1.36	1.36–1.46	1.57	1.49–1.65
Upper jaw length on blind side (UJBL)	1.39	1.41–1.48	1.61	1.52–1.71
Mandible length on ocular side (MOL)	1.06	1.04–1.11	1.16	1.14–1.19
Mandible length on blind side (MBL)	1.06	1.04–1.10	1.16	1.14–1.19
Upper eye diameter (UED)	4.10	3.22–3.71	4.46	3.80–4.71
Lower eye diameter (LED)	4.37	3.37–4.06	4.46	3.80–4.71
Interorbital width (IOW)	10.61	9.49–10.97	7.25	6.60–9.50
Pectoral-fin length on ocular side (PEOL)	1.84	1.67–1.83	2.15	1.72–2.15
Pectoral-fin length on blind side (PEBL)	4.20	2.66–3.72	3.41	2.62–3.41
Pelvic-fin length on ocular side (PLOL)	2.98	2.48–2.71	2.64	2.29–2.85
Pelvic-fin length on blind side (PLBL)	3.37	2.71–3.53	2.90	2.58–3.17
Lateral-line curve length on ocular side (LCL)	1.78	1.59–1.98	1.93	2.12
Lateral-line curve depth on ocular side (LCD)	8.48	6.67–8.32	NA	NA
Caudal peduncle depth (CPD)	3.96	3.79–4.06	4.46	3.93–5.17
Meristic counts				
Dorsal-fin rays	109	108–114	112	106–116
Anal-fin rays	78	77–84	82	80–85
Pectoral-fin rays on ocular side	16	14–16	15	14–17
Pectoral-fin rays on blind side	15	13–15	15	13–16
Caudal-fin rays	2+13+2	2+13+2	4+9+3	4+10+3
Gill rakers on ocular side	0+14	0+14–18	0+15	0+13–19
Vertebrae	16+38=54	16+36–37=52–53	16+38=54	16-17+34-39=50-56
Lateral-line scales	155	137–167	160	156-181
Pseudobranches	22	21–25	NA	26-27
Reference	This study		Foroshchuk 1991	

Radiograph. Second neural spine on the thoracic vertebra inserted between the 16th–17th (15th–16th in the holotype) interneural bones of the dorsal fin. First haemal spine on the caudal vertebra is inserted between the 10th–11th (holotype), 11th–12th, and 12th–13th (paratypes) interhaemal bones of the anal fin (Fig. 2C).

Etymology. The name of the new species refers to the type locality in Papua New Guinea where it is likely endemic.

Distribution. *Chascanopsetta novaeguineae* **sp. nov.** is known only from the northern shelf at depths of 273–505 m off New Hanover Island which is located at northern part of the Bismarck Archipelago of Papua New Guinea (Fig. 1).

Biology. The radiograph showed two small fishes in the holotype's stomach, which indicates that *C. novaeguineae* **sp. nov.** seems to be piscivorous (Fig. 2C).

Remarks. Two non-types (ASIZP 73836 and NTUM 10990) are juveniles and the morphological examinations of these specimens showed substantial morphometric differences compared with those of adults (Supplementary Material Table 1). Their genetic differences compared to adult specimens are negligible, which confirms their status as belonging to the same species. Morphometric differences may indicate the ontogenetic variation in these features between juveniles and adults. We restricted the intra-specific range of morphometric characters reported by excluding the data of these juveniles. Therefore, these two specimens were excluded from the type series in the species description.

TABLE 3. Matrix of inter-specific genetic divergences (measured by K2P-distances) at the *COI* locus, deduced from the nucleotide sequences sampled in four species of the genus *Chascanopsetta*.

Sample		Sample			
No.	Species	1	2	3	4
1	*C. novaeguineae* **sp. nov.**	-			
2	*C. danae*	0.125	-		
3	*C. prognatha*	0.125	0.077	-	
4	*C. lugubris*	0.159	0.159	0.164	-

Molecular results

The ML phylogenetic tree inferred based on the *COI* gene dataset resolves the monophyly of the genus *Chascanopsetta* with maximum support (Fig. 5). Among the species within the genus that were analyzed, three well-supported lineages (bootstrap proportion [BP] >90%) are presented (Fig. 5). The first lineage comprises two species (*C. danae* and *C. prorigera*), the second lineage includes all of the samples of *C. novaeguineae* **sp. nov.,** and the third lineage contains the specimens of the widely distributed species *C. lugubris*. *Chascanopsetta novaeguineae* **sp. nov.** is a sister species to the first lineage (*C. danae* and *C. prorigera*) with moderate support (BP = 65%).

Genetic differences (measured by K2P distance) at the *COI* locus between the new species and other congeners (*C. danae, C. prorigera,* and *C. lugubris*) are 12.5%, 12.5%, and 15.9%, respectively (Table 3). The average genetic divergence among species in the surveyed genus *Chascanopsetta* is much higher than that (9.93%) reported from a DNA *COI* barcoding study with more than 200 marine fishes surveyed (Ward *et al.* 2005), and is also higher than that (8.29%) of congeneric individuals of almost 200 species inventoried among the Canadian freshwater fish fauna (Hubert *et al.* 2008).

Discussion

Chascanopsetta novaeguineae and *C. elski* are among the four species of *Chascanopsetta* with higher counts of gill rakers on the lower part of the first gill arch, and have more than 13 gill rakers. However, *C. novaeguineae* differs from *C. elski* in having fewer pseudobranches (21–25 vs. 26–27) (Table 2). Moreover, the differences between these two species are also evident in several morphometric measurements. For example, *C. novaeguineae* has a narrow interorbital width (ratio of HL to IOW 9.49–10.97 vs. 6.60–9.50 in *C. elski*); larger jaws on both sides of the body (ratio of HL to UJOL 1.36–1.46 vs. 1.49–1.56 in *C. elski*; ratio of HL to UJBL 1.39–1.48 vs. 1.52–1.71 in *C. elski*); and longer lower jaw length on both sides (ratio of HL to MOL 1.04–1.11 vs. 1.14–1.19 in *C. elski*; ratio of HL to MBL 1.04–1.10 vs 1.14–1.19 in *C. elski*). In addition, *C. novaeguineae* differs from *C. elski* in having a much shorter blind-side anterior nostril and in having the posterior part of the anterior nostril flap not reaching the base of the posterior nostril (vs. longer blind-side anterior nostril reaching base of posterior nostril in *C. elski*).

Foroshchuk (1991) described *C. elski* as having the neural spine of the second thoracic vertebrae placed between the 16th–17th interneural bones of the dorsal fin and the haemal spine on the first caudal vertebra placed between the 11th–13th proximal pterygiophores of the anal fin. No differences in placements of these osteological features were observed between our new species and *C. elski* (see Fig. 2C and the description shown above). Based on these similarities, these osteological features may not be diagnosable for all species within the genus.

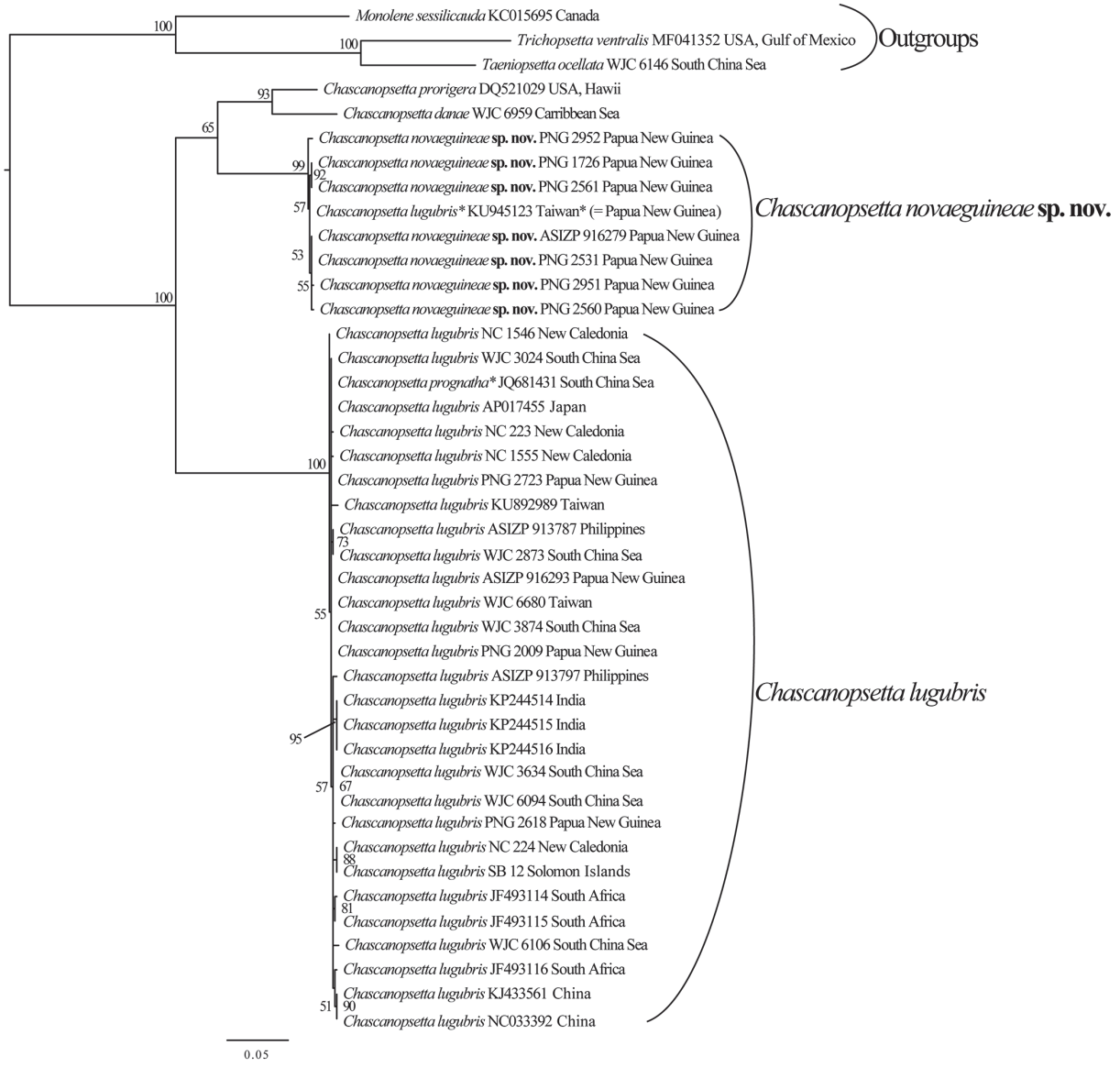

FIGURE 5. Maximum-likelihood (ML) tree based on DNA sequences of the partial *COI* gene. Numbers on nodes are bootstrap (BP) values in percentage in the ML analysis. Values below 50% not shown. Sample numbers can be referenced in Table 1. Erroneous sequences (see discussion) from Genbank are marked with asterisk.

Most species in the genus *Chascanopsetta,* except *C. lugubris,* have been reported from restricted geographic areas (Fig. 1). For example, in the western Indian Ocean, *C. kenyaensis* is distributed from Kenya to southern Somalia, while *C. elski* is found only at the Saya de Malha Bank (Foroshchuk 1991; Hensley & Smale 1997). According to our results, the distribution of *C. novaeguineae* is probably restricted to the West Pacific, as it is known only from sites off Papua New Guinea. These sites are located about 10,000 kilometers from the reported sites of occurrence of its similar congener, *C. elski.* Allopatric distributions of such similar species may indicate that physical isolation over a very great geographic distance consequently led to reproductive isolation and speciation resulting in two species.

Chascanopsetta lugubris sensu lato is the only *Chascanopsetta* member with an extremely wide range distribution throughout tropical waters from the Atlantic Ocean to the Indo-Pacific (Amaoka & Yamamoto 1984; Munroe 2003). Bruun (1937) described a subspecies, *C. lugubris danae,* from a single specimen collected from off the West Africa at a depth of 1500 m. Amaoka (1969) considered that *C. lugubris danae* is a junior synonym of *C.*

lugubris. Later, Amaoka & Parin (1984) examined specimens collected from the Atlantic Ocean, and concluded that these specimens should be recognized with the subspecies designation, *C. lugubris danae sensu* Bruun 1937. They provided an identification key for the two subspecies. Hensley & Smale (1997) examined otolith features of three nominal taxa (*C. kenyaensis*, *C. lugubris lugubris*, and *C. lugubris danae*), and found that the otoliths of *C. kenyaensis* were twice the diameter of those of both *C. lugubris lugubris* and *C. lugubris danae*. Furthermore, they found no significant differences in the otolith morphology between those of *C. lugubris lugubris* and *C. lugubris danae*. Munroe (2003) regarded *C. danae* as a valid species based on its higher gill-raker counts (4–8) on the lower limb of this species than compared with that of *C. lugubris lugubris* (0–5). In the present study, the genetic divergence between these two nominal taxa (*C. lugubris* from South Africa, India, Japan, Philippines, Papua New Guinea, Taiwan, New Caledonia, and Solomon Islands and *C. danae* from the Caribbean Sea) showed that the *COI* sequences of these two nominal taxa differed by 15.9%. Our molecular results, therefore, support the taxonomic classification proposed by Munroe (2003), and indicate that individuals of *C. lugubris* occurring in the Caribbean Sea and adjacent areas of the Atlantic should be referred to as *C. danae*. However, the description of *C. danae* given by Munroe (2003) differs with the originally description of Bruun (1937). The holotype of *C. danae* was originally collected off West Africa in deeper waters compared to those where other congeners had been collected (Bruun 1937). Munroe (2003) described *C. danae* based on specimens collected from the western central Atlantic at depths of 160 to 460 m. More specimens and molecular analysis are needed to resolve this taxonomic issue regarding the status of the eastern Atlantic specimens of *Chascanopsetta*.

Some misidentifications are found in the sequences deposited in GenBank identified as different species of *Chascanopsetta*. For instance, the sequence KU945123 reported in Chang *et al.* (2017) was associated with the specimen (specimen voucher: ASIZP 73836; tissue voucher: ASIZP 916333) collected from "Taiwan" and originally identified as "*C. lugubris"*. However, according to the results of our molecular analysis, the sequence grouped with those from *C. novaeguineae* (Fig. 5). After re-examination of the voucher specimen deposited in the ASIZP ichthyological collections and checking the collection information of the specimen, we confirmed that it is a juvenile of *C. novaeguineae* collected off Papua New Guinea, and not from off Taiwan. Another likely case with specimen misidentification in GenBank is the sequence JQ681431 (sample collected from the South China Sea). This sequence was associated with a name "*C. prognatha*" but emerged within the *C. lugubris* clade in the *COI* gene tree (Fig. 5).

In the present study, we conducted both morphological and molecular approaches to support the description of *Chascanopsetta novaeguineae*. Even though DNA sequence data from the most morphologically similar species (*C. elski*) were not examined (no fresh samples were available for sequencing), morphological examination provides clear distinguishing characters to discriminate between these two species. Previous hypotheses grouped species of *Chascanopsetta* into two groups, including a group with high counts for gill rakers and another characterized in having low counts of gill rakers. The new species has a higher number of gill rakers on the lower part of the first gill arch and appears to be sister to the clade comprising *C. prorigera* and *C. danae*, species that previously were classified in the high and low gill raker counts species groups, respectively. The preliminary molecular results presented herein indicate that the two previously hypothesized species groups of *Chascanopsetta* featuring either high or low gill raker counts do not represent reciprocally monophyletic groups. Results of this study also provide initial insights into the species phylogeny and need for taxonomic revision of the genus *Chascanopsetta*. Additional specimens of different species of *Chascanopsetta* and specimens from geographically diverse regions should be examined by morphological and molecular analyses to gain more knowledge about the systematics of this bothid genus.

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SUPPLEMENTARY MATERIAL TABLE 1. Meristic features and morphometric measurements of the non-types of *Chascanopsetta novaeguineae* **sp. nov.**.

	ASIZP 0073836	NTUM 10990
Standard Length, SL (mm)	104.73	115.82
Ratio of SL to		
Head length (HL)	4.58	4.57
Body depth (BD)	3.15	3.62
Ratio of HL to		
Snout length (SNL)	4.35	4.77
Upper jaw length on ocular side (UJOL)	1.48	1.39
Upper jaw length on blind side (UJBL)	1.51	1.48
Mandible length on ocular side (MOL)	1.19	1.07
Mandible length on blind side (MBL)	1.18	1.09
Upper eye diameter (UED)	4.20	3.25
Lower eye diameter (LED)	3.92	3.30
Interorbital width (IOW)	11.22	12.38
Pectoral-fin length on ocular side (PEOL)	2.00	2.09
Pectoral-fin length on blind side (PEBL)	3.83	NA
Pelvic-fin length on ocular side (PLOL)	NA	NA
Pelvic-fin length on blind side (PLBL)	2.73	NA
Lateral-line curve length on ocular side (LCL)	2.16	1.99
Lateral-line curve depth on ocular side (LCD)	7.65	10.48
Caudal peduncle depth (CPD)	3.61	4.33
Meristic counts		
Dorsal-fin rays	118	111
Anal-fin rays	81	82
Pectoral-fin rays on ocular side	15	15
Pectoral-fin rays on blind side	14	16

.....continued on the next page

SUPPLEMENTARY MATERIAL TABLE 1. (Continued)

	ASIZP 0073836	NTUM 10990
Caudal-fin rays	2+13+2	2+13+2
Gill rakers on ocular side	0+18	0+16
Vertebrae	16+37	16+37
Lateral-line scales	148	154
Pseudobranches	NA	NA
Reference	This Study	